Study Title: Optimising the dose of tafenoquine for the radical cure of *Plasmodium vivax* malaria in Southeast Asia

Short Title: Southeast Asia Dose Optimisation of Tafenoquine (SEADOT)

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Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, Data Safety Monitoring Board, host institutions, regulatory authorities, and members of the relevant ethics committees, unless authorised to do so.

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LIST OF ABBREVIATIONS

AE	adverse event
AL	artemether-lumefantrine
AR	adverse reaction
AUC	area under the curve
CBC	complete blood count
CQ	chloroquine
CRF	case report form
CYP	cytochrome P450
DP	Dihydroartemisinin piperaquine
DSMB	Data Safety Monitoring Board
EDTA	ethylenediaminetetraacetic acid
G6PD	glucose-6-phosphate dehydrogenase
GCP	good clinical practice
HCG	human chorionic gonadotropin
ICF	informed consent form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committees
IRB	Institutional Review Board
LOMWRU	Lao Oxford Mahosot Hospital Wellcome Trust Research Unit
MetHb	methaemoglobin
MORU	Mahidol Oxford Tropical Medicine Research Unit
MVRU	Mahidol Vivax Research Unit
OUCRU	Oxford University Clinical Research Unit
OxTREC	Oxford Tropical Research Ethics Committee
PD	pharmacodynamic
PI	principal investigator
PID	patient identification
PK	pharmacokinetic
PQ	primaquine
P. vivax	*Plasmodium vivax*
RBCs	red blood cells
SAE	serious adverse event
SAR	serious adverse reaction
SID	study identification number
SMRU	Shoklo Malaria Research Unit
SOP	standard operating procedure
SpMet	transcutaneous methaemoglobin measurement
$t_{1/2}$	elimination half-life
T_{max}	time of peak drug concentration
TQ	tafenoquine
WBC	white blood cell
WHO	World Health Organization

1. STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with the International Conference on Harmonisation (ICH) E6 and the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46). The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approvals from the Independent Ethics Committees (IECs), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

Principal Investigator (PI):

Signature: _____ Date: _____

Print Name: _____

2. PROTOCOL SUMMARY

Study Title	**Optimising the dose of tafenoquine for the radical cure of *Plasmodium vivax* malaria in Southeast Asia**
Study Design	Double-blind randomised superiority trial
Phase	Phase 4
Background	Tafenoquine was recently approved by regulatory authorities in the USA and Australia. Tafenoquine is an alternative radical curative treatment to primaquine acting against the dormant liver stage of *Plasmodium vivax* (the hypnozoite). Tafenoquine (an 8-aminoquinoline) has the substantial advantage of single dosing as compared to a 14-day course of primaquine to achieve radical cure. The recommended tafenoquine dose is 300 mg, which was shown to be significantly worse in radical curative efficacy to a total primaquine dose of 3.5 mg/kg in Southeast Asia. The cure rate of tafenoquine 300 mg in Southeast Asian study sites was only 74%. The comparator 3.5 mg/kg total primaquine dose is the standard and most commonly used dose globally, but in Southeast Asia and the Western Pacific, higher doses of primaquine are needed for radical cure. This study aims to determine the optimal dose of tafenoquine in Southeast Asia. Addendum for Indonesia: The INSPECTOR trial results showed that tafenoquine 300mg was not efficacious for radical cure (79% probability for recurrence after treatment). The comparator arm, low-dose primaquine 3.5mg/kg divided equally over 14 days, showed a 48% probability of recurrence after treatment). The standard of care for radical cure in Indonesia is high-dose primaquine 7mg/kg divided in 7 daily doses).
Objectives	*Primary Objective:* • Determine whether a 50% increase in tafenoquine dose (TQ-higher) provides better efficacy than the currently recommended tafenoquine dose (TQ-current) as measured by the absence of recurrent *P. vivax* infection by 4 months • Determine whether TQ-higher provides better efficacy to high-dose (7mg/kg total dose) primaquine as measured by the absence of recurrent *P. vivax* infection up to month 4 in Indonesia only. *Secondary Objectives:* • Determine whether a 50% increase in tafenoquine dose (TQ-higher) provides greater clearance of sub-microscopic *P. vivax* infection than the currently recommended tafenoquine dose (TQ-current) • Characterise the safety and tolerability of a 50% higher tafenoquine dose (TQ-higher) • Determine the predictive utility of day 7 methaemoglobin for tafenoquine radical cure • Characterise TQ population PK and PD and correlate the *CYP2D6* metaboliser status and genotype with TQ concentrations *in vivo* • Explore the presence of TQ metabolites *in vivo* • Characterise the best predictors of recurrence at day 28 and month 4 • Assess the utility of a day 14 and 21 ultrasensitive qPCR parasite quantitation as a predictor of subsequent relapse

	• Determine whether TQ-current provides better efficacy to high-dose (7mg/kg total dose) primaquine as measured by the absence of recurrent *P. vivax* infection up to month 4 in Indonesia only.
Endpoints	*Primary Endpoint:* • Microscopy positive *P. vivax* recurrence after radical cure treatment up to month 4 *Secondary Endpoints:* • Sub-microscopic *P. vivax* recurrence after radical treatment between day 28 and month 4 • Sub-microscopic *P. vivax* recurrence with very low parasite density after radical cure treatment at days 14 and 21 • Number of serious adverse events (e.g., hospitalisation for symptomatic haemolysis or metheamoglobinaemia) • Number of adverse events (e.g., gastrointestinal symptoms) • Methaemoglobin levels measured by pulse oximetry at day 7 • Tafenoquine AUC and $t_{1/2}$ as estimated from plasma tafenoquine concentrations at days 0, 1, 2, and 7, and monthly visits until month 4 • Plasma tafenoquine concentrations at days 0, 1, 2, and 7, and at monthly visits until month 4 • Tafenoquine metabolites identification in red blood cell and urine at days 1, and 2 or 3. • The probability of treatment failure as defined by the probability of one or more of the observed recurrences within 4 months being a relapse, using time-to-event and estimated identity-by-descent of recurring parasite genotypes compared to enrolment parasite genotypes • Microscopy positive *P. vivax* recurrence after radical cure treatment at months 1, 2, 3, and 4
Population	Patients (adults and children) with *Plasmodium vivax* mono-infection who present for consultation with a fever or history of fever
Sample size	A total of 700 participants (350 per arm) will be recruited, with competitive enrolment across participating countries until the target sample size is reached. Applicable only for Indonesia: to assess background efficacy of radical cure using the standard of care primaquine regimen, 120 participants are added for an additional arm (DP+PQ7-high) which increases the total participants in this trial to 820.
Inclusion and Exclusion Criteria	Inclusion • Patients with symptomatic *P. vivax* mono-infection as diagnosed by microscopy • Fever or history of fever in the previous 7 days • Quantitative G6PD activity ≥70% of the population median • Weight >10 kg and ≥2 years old • Ability to understand the study instructions and provide written informed consent.

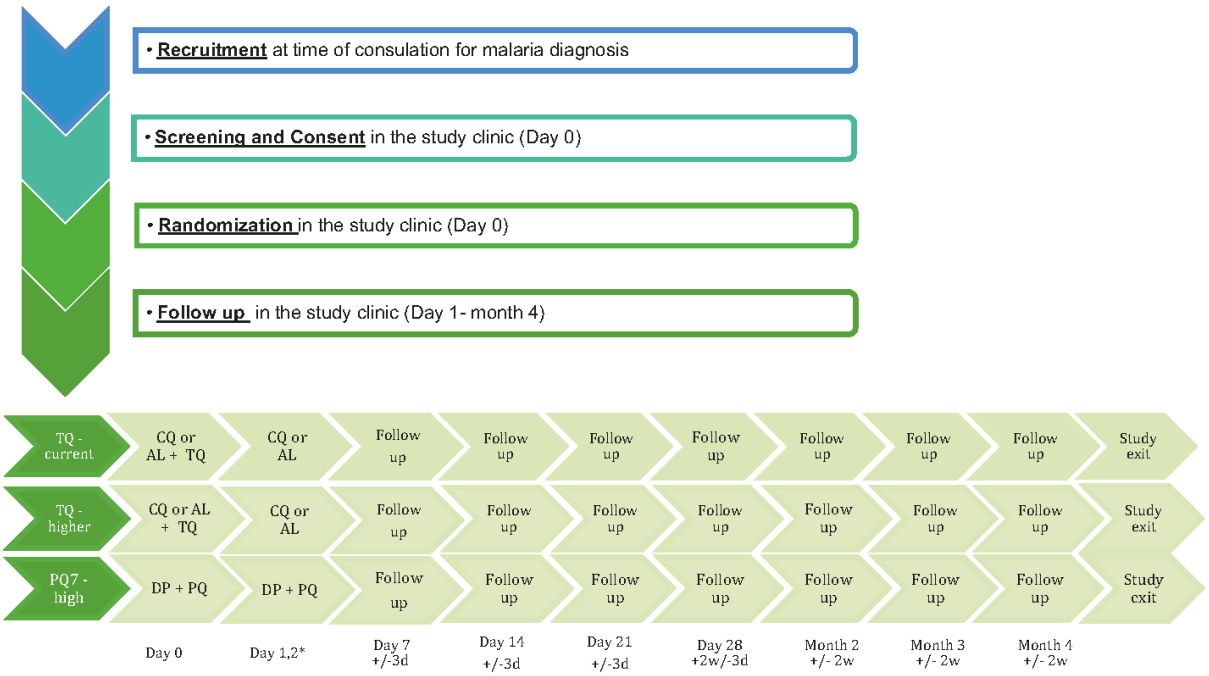
	• Willing to be followed for 4 months Exclusion • Pregnancy • Lactation • Hb < 8 g/dL • Severe malaria • Blood transfusion in the last 4 months • History of allergic response to an 8-aminoquinoline or the nationally recommended schizonticide (e.g., chloroquine, artemether-lumefantrine) • Any previous history of a haemolytic event • Presence of any condition which in the judgement of the investigator would place the patient at undue risk or interfere with the results of the study (e.g. chronic disease, medications that potentiate or inhibit *CYP2D6* or *CYP2C8* isoenzyme function)
Number of participating countries and organisation involved	Cambodia – Mahidol Oxford Tropical Medicine Research Unit (MORU) Lao PDR – Lao Oxford Mahosot Hospital Wellcome Trust Research Unit (LOMWRU) Thailand – Shoklo Malaria Research Unit (SMRU) and Mahidol Vivax Research Unit (MVRU) Vietnam – Oxford University Clinical Research Unit (OUCRU) Indonesia – Oxford University Clinical Research Unit (OUCRU) at the Faculty of Medicine Universitas Indonesia (FMUI)

Description of Study Drug:	Schizonticidal drug:
	• Cambodia, Thailand, Vietnam and Indonesia: Chloroquine (250 mg tablets) 25 mg/kg base divided over 3 days (D0, 1, 2)
	• Lao PDR: Artemether-lumefantrine (20/120 mg tablets) 80 mg/480 mg twice daily for 3 days (D0, 1, 2)
	• Indonesia: Dihydroartemisinin piperaquine (40/320 mg tablets) weight ≥60 kg 4 tablets once daily for 3 days (D0, D1, D2), weight <60kg 3 tablets once daily for 3 days (D0, D1, D2)
	Radical curative agent:
	• All sites: randomisation into 2 single dose tafenoquine (TQ) arms
	  ○ TQ-current (currently recommended tafenoquine dose)
	  ○ TQ higher (a 50% increase from the currently recommended tafenoquine dose
	• Indonesia site:
	  ○ PQ (standard 7.0mg/kg divided in 7 daily doses)

Study Duration	5 years (estimated Sep 2023 to Dec 2027). Recruitment will be performed in 3-4 years with 4-month follow-up. Study preparation will take ~6 months before study start. Laboratory and data analysis will take 6 months after the study is completed.
Participant Duration of Follow-up	4 months

3. SCHEMATIC OF STUDY DESIGN

Figure 1. Study Visit Flow Chart



*Daily follow up will continue until the malaria smear is negative (for asexual stages of *P. vivax*) one time.

Note: The schizonticidal drug used will depend on the country national recommendations for *P. vivax* treatment; Cambodia, Thailand, Indonesia and Vietnam: chloroquine; Lao PDR: artemether-lumefantrine; Indonesia: dihydroartemisinin-piperaquine. Applicable only for Indonesia (from the INSPECTOR trial results): a hypnozoiticidal drug arm is included, primaquine 7.0 mg/kg divided in 7 daily doses.

4. KEY ROLES

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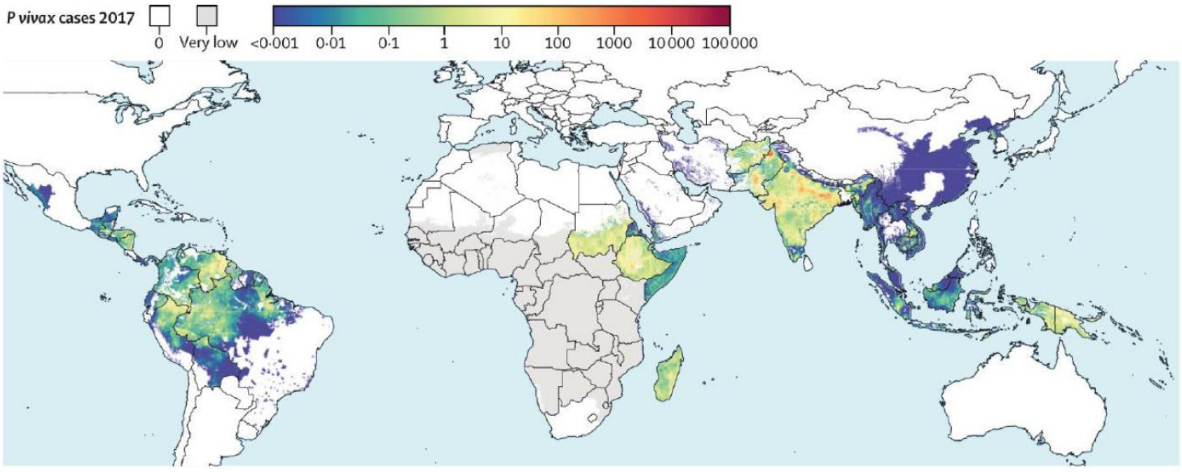
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5. INTRODUCTION

5.1 Background Information

Whilst the global burden of *Plasmodium vivax* malaria has declined since 2010, *Plasmodium vivax* is still estimated to have caused 14.3 million symptomatic cases of malaria in 2017 (1) (Figure 2). Of these, over half (7.3 million) are in the Southeast Asian region (1). In this densely populated region, short latency *P. vivax* parasites predominate and result in frequent infections as close as 3-4 weeks apart. Relapses are a major cause of infection and morbidity (2–4) and in higher transmission settings contribute to severe anaemia and death in young children.

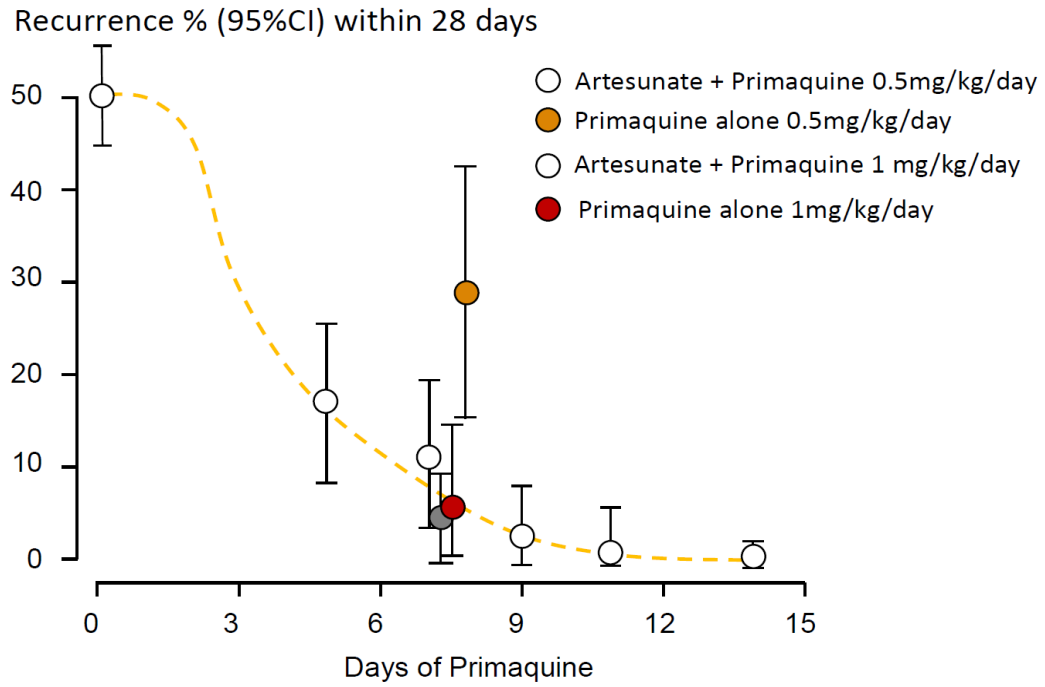
Figure 2. Number of *Plasmodium vivax* cases globally



SE Asia has the highest proportion of the estimated global burden of *P. vivax* cases: 51.2% (7·3 million of 14·3 million) in 2017.

In SE Asia over half of the patients treated for an acute *P. vivax* blood stage infection will develop at least one relapse (5–7) if an anti-relapse drug is not given. The only widely available drug to prevent relapses (radical cure) is the 8-aminoquinoline primaquine. The doses of primaquine required to prevent relapses in Southeast Asia and the Western Pacific region are higher than elsewhere. (Figure 3) *P. vivax* relapses more in these regions where with large populations over 80% of the global *P. vivax* burden occurs so it is likely that most of the world's relapses occur in East Asia and thus the benefits of effective radical cure are greatest in this area.

Figure 3. Recurrence (re-infection, relapse or recrudescence) rates of *Plasmodium vivax* after radical cure



There are several obstacles to widespread use of primaquine, which is why primaquine is widely recommended but not widely used. Most important is the need to test for G6PD deficiency, a common genetic defect in malaria endemic areas. In resource limited settings where G6PD testing is not available, primaquine is often not prescribed. This is because 8-aminoquinolines have long been known to cause haemolysis (red blood cell breakdown) in persons who have G6PD deficiency (low levels of erythrocyte G6PD activity) (8,9). Haemolysis following primaquine (and other oxidant drugs (10)) occurs in all people who are G6PD deficient (11–13). The degree of haemolysis varies markedly depending on many factors, but most importantly the degree of G6PD deficiency and the extent of drug exposure (14).

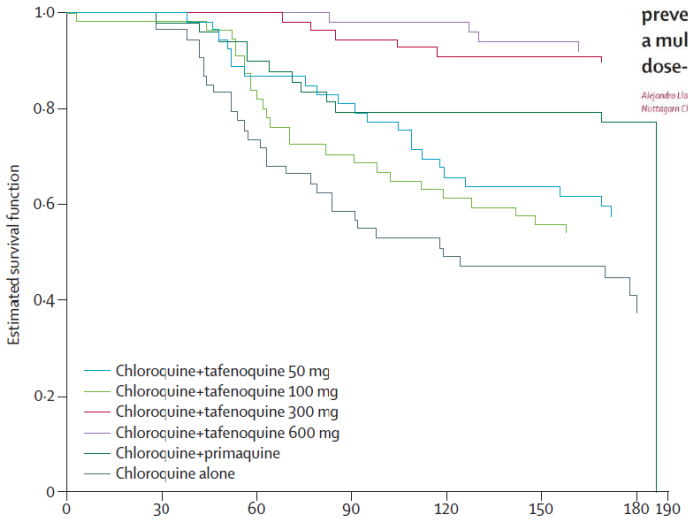
Adherence to the currently recommended 14-day primaquine regimen is another challenge to widespread primaquine use and efficacy (15–19). A short course regimen of primaquine using the same doses but given over 7 days (total adult dose of either 3.5 or 7mg/kg depending on country) (20,21) may improve adherence. The high dose short course primaquine does not appear to be safe in G6PD intermediate individuals (12).

Recently a slowly eliminated 8-aminoquinoline, tafenoquine, has become available. This can be given in a single dose (22–24) which removes completely the issue of low effectiveness because the single dose can be supervised. However, tafenoquine is contraindicated in persons with G6PD activity below 70% (13). This is a serious limitation as *qualitative* screening tests (e.g. lateral flow rapid diagnostic G6PD tests) fail to identify G6PD intermediate individuals (G6PD activity 30-70%). Thus, *quantitative* G6PD deficiency testing is required to identify individuals with intermediate G6PD

activity and this population (female heterozygotes) may experience significant haemolysis with tafenoquine (13). Near patient quantitative G6PD tests such as the G6PD Biosensor devices (25,26) are now available for use before tafenoquine is prescribed. A large usability trial in health clinics with over 1,700 participants in Brazil showed the quantitative STANDARD G6PD test was 100% sensitive for G6PD deficient (<30% activity) individuals (27). Thus, this test is appropriate for determining G6PD status and eligibility for tafenoquine.

In dose ranging studies of tafenoquine, the maximum radical curative efficacy was obtained at an adult single dose of 600 mg (28) (Figure 4). Because of slightly better tolerability a dose of 300 mg was chosen for further evaluation and in 2018 this was approved by US FDA and the Australian regulatory authority (22). Regulatory approval in Brazil and Thailand have followed in 2019 and 2020; respectively.

Figure 4. Radical curative efficacy of tafenoquine in a dose ranging study



Tafenoquine plus chloroquine for the treatment and relapse prevention of *Plasmodium vivax* malaria (DETECTIVE): a multicentre, double-blind, randomised, phase 2b dose-selection study

Alejandro Llanos-Cuentes, Marcus V Lacerda, Ronnatrai Rueangweerayut, Srivicha Krudsood, Sandeep K Gupta, Sanjoy K Kochar, Preetam Arthur, Nuttagarn Chuenchom, Jörg J Möhrle, Stephan Duparc, Cletus Ugwuegbulam, Jörg-Peter Kleim, Nick Carter, Justin A Green, Lynda Kiffam

Lancet 2014; 383: 1049–58

Number at risk						
Chloroquine +tafenoquine 50 mg	54	46	43	34	32	8
+tafenoquine 100 mg	55	46	38	33	30	7
+tafenoquine 300 mg	56	56	53	50	50	18
+tafenoquine 600 mg	55	53	50	49	46	11
+primaquine	49	44	37	37	37	11
Chloroquine alone	52	39	31	26	24	11

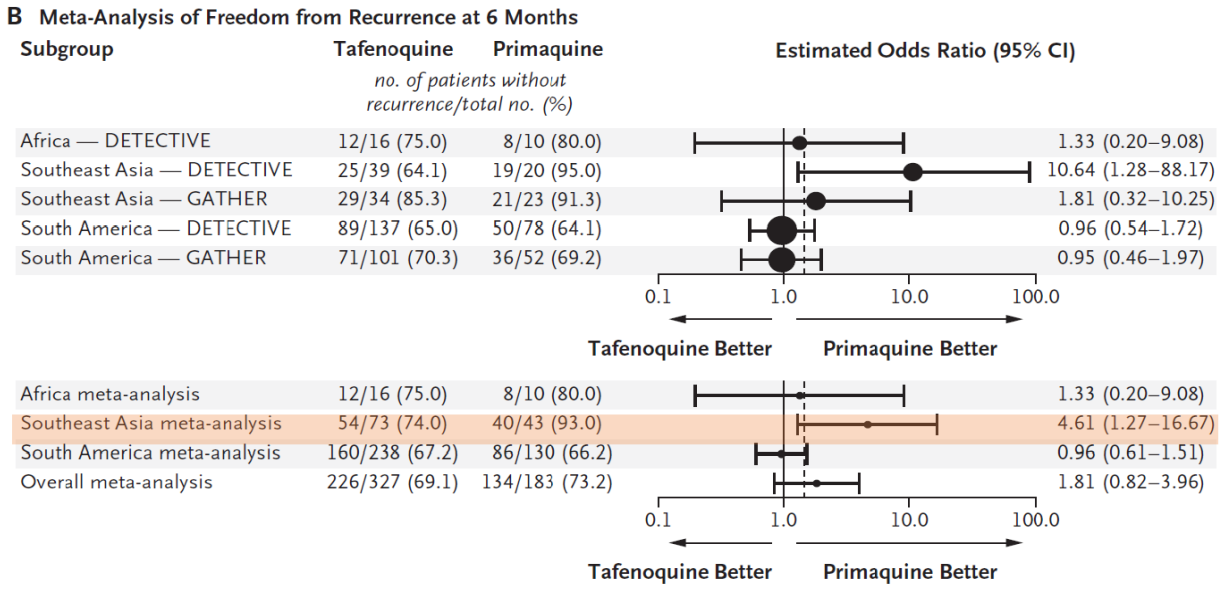
Ethnic origin						
American Indian	27 (49%)	28 (49%)	29 (51%)	29 (52%)	25 (50%)	27 (50%)
Asian, central/south	11 (20%)	11 (19%)	9 (16%)	10 (18%)	6 (12%)	10 (19%)
Asian, southeast	16 (29%)	16 (28%)	19 (33%)	16 (29%)	16 (32%)	16 (30%)
Mixed	1 (2%)	2 (4%)	0	1 (2%)	3 (6%)	1 (2%)

The 300 mg dose of tafenoquine however, proved significantly inferior to low dose primaquine in a meta-analysis of the phase 3 studies when restricted to the Southeast Asian region (23,24). These data strongly suggest that a higher dose of tafenoquine is needed in the Southeast Asian and Western Pacific regions (Figures 5). Results from the INSPECTOR trial in Indonesian soldiers returning to non-endemic areas showed low relapse-free rates of the single 300mg dose tafenoquine

combination with dihydroartemisinin-piperaquine (21%) similar to dihydroartemisinin-piperaquine alone (11%) (29). This has prompted a product label update for Krintafel™ (the branded form of tafenoquine in the USA) that tafenoquine should be combined only with chloroquine and not with other antimalarials e.g. artemisinin-based combination therapies (ACT) (30). This prevents the use of tafenoquine in areas with chloroquine resistant *P. vivax* parasites where national malaria programmes recommend ACTs for vivax malaria.

In areas with chloroquine resistance, such as eastern Indonesia, the possibility of a higher dose of tafenoquine combined with dihydroartemisinin has not been evaluated. Moreover, tafenoquine combined with chloroquine cleared asexual parasitemias of the chloroquine-resistant AMRU-1 strain of *P. vivax* in a non-human primate model (31), raising the possibility of efficacious radical cure of *P. vivax* using tafenoquine combined with chloroquine despite resistance to chloroquine. In the 20 years since the first clinical trial of tafenoquine was conducted in humans (28), higher doses of tafenoquine have been evaluated but only in a small number of studies. A 400 mg dose of tafenoquine has been evaluated in five different studies (32–36) . All of them used a loading dose of 400 mg daily for 3 days (total dose 1200 mg), mostly as an indication for malaria prophylaxis. One of these studies by Fukuda et al. assessed the schizonticidal (blood stage) and radical curative efficacy against *P. vivax*, whereas the other four assessed prophylaxis. A total dose of 1200 mg was 100% efficacious against recurrences at day 120 (36) (Table 1).

Figure 5. Meta-analysis results for the non-inferiority of tafenoquine 300 mg compared to low dose primaquine 0.35 mg/kg



Low dose primaquine (0.35mg/kg) is better than tafenoquine (0.5mg/kg) in SE. Asia

26% recurrence rate within 6 months

Table 1. Studies assessing a 400 mg dose of tafenoquine

Authors	Year	Indication	TQ dosing regimen	Methaemoglobinaemia	Abd pain
Shanks, GD et al	2001	causal prophylaxis	400mg/day x 3d or 200mg/day x 3d, then weekly at same dose	Not reported	dose dependent symptoms
Nasveld, P et al	2002	PART	400mg/day x 3d or 200mg twice daily x 3d	Not reported	more than PQ arm
Walsh, DS et al	2004	causal prophylaxis	400mg/day x 3d then monthly	Elevation highest after loading dose	more than PQ arm
Elmes, NJ et al	2008	PART	400mg/day x 3d or 200mg twice daily x 3d or 200mg/day x 3d	Not reported	dose dependent symptoms
Fukuda M, et al	2017	Schizonticide and Radical cure	400mg/day x 3d	Mean Methb increase was 9%	similar to PQ arm

PART – presumptive anti-relapse treatment, TQ – tafenoquine, PQ – primaquine

In radical curative efficacy studies (all included Southeast Asian populations), single dose tafenoquine 600 mg had a radical curative rate >91% against *P. vivax* (22,37). In these studies, the fixed dose resulted in a weight-based range of 3.6 – 14.3 mg/kg/dose. In comparison, when a single dose of tafenoquine 300 mg was given, the radical curative rate was 64-89% (23,24), and the resulting weight-based range was 2.2 – 8.0 mg/kg/dose. Recent results from the first pharmacokinetic, safety and efficacy analysis of tafenoquine in children ages 2-15 years with *P. vivax* malaria showed that a 50, 100, 200, and 300 mg dose based on the 4 weight bands ≥5 to ≤10 kg, > 10 to ≤ 20 kg, > 20 to ≤ 35 kg, and ≥ 35 kg, respectively, achieved the target $AUC_{0-\infty}$ with no recurrences or safety issues (38). However, more data are required to confirm the TEACH trial findings especially at the lower weight bands.

To characterise the relationship of tafenoquine dose and radical cure efficacy we obtained all the individual participant data (N=1102) from the Phase 3 pre-registration trials (DETECTIVE parts 1 and 2; and GATHER) (22–24) and performed a pharmacometric meta-analysis. The results show that:

a) The dose response curve is particularly steep around the currently recommended dose (~5mg/kg). (Figure 6)
b) [*RC1*] The odds ratio for *P. vivax* recurrence (<4 months) is 0.69 (95% CI 0.64 to 0.75; $p=10^{-21}$) for each mg/kg increase in tafenoquine dose. (Figure 7)
c) Tafenoquine radical curative activity correlates independently with dose (but not parent drug AUC) and also with methemoglobinemia and faster drug elimination (proof of the central pharmacological role of oxidative metabolites).

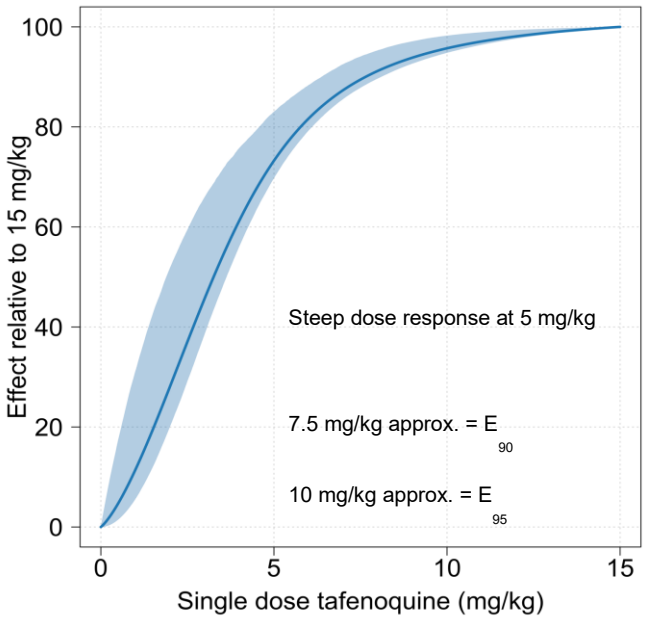


Figure 6. Dose response curve of tafenoquine. E_{max} model allows for plateauing of effect which is more biologically plausible

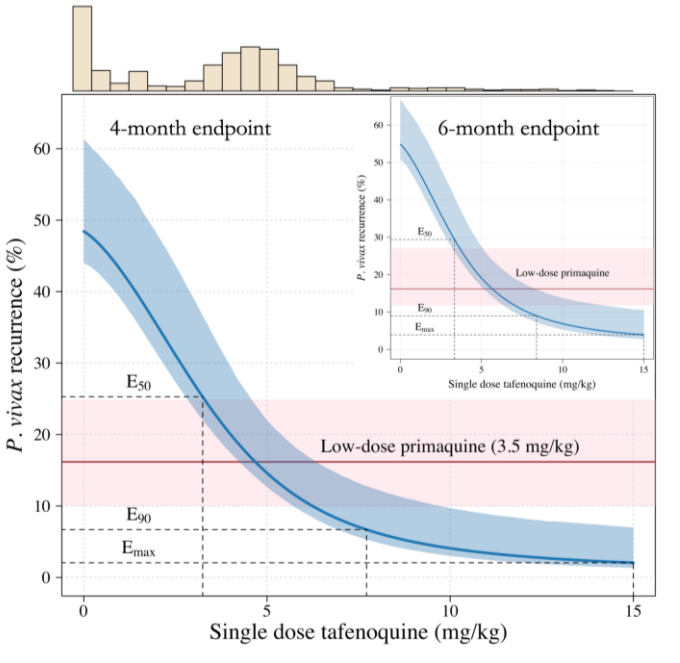


Figure 7. The relationship of weight adjusted tafenoquine dose and *P. vivax* recurrence at 4 and 6 months

Thus, increasing tafenoquine from the current dose (e.g., 300mg adult dose) to a 50% higher dose (e.g. 450mg adult dose) is predicted to more than halve the relapses and result in an overall >90% reduction of *P. vivax* recurrence (39).

Similar to primaquine, the gastrointestinal adverse effects of tafenoquine have been shown to be dose dependent (40). Gastrointestinal symptoms were lessened if tafenoquine was taken with food. Overall, in detailed prospective studies tafenoquine doses up to 10mg/kg have proven to be well tolerated.

This double blind randomised superiority study in Southeast Asia aims to compare the anti-relapse efficacy of the currently recommended tafenoquine dose (TQ-current) and a dose which is 50% higher (TQ-higher); e.g. 300mg versus 450mg in an adult. A single fixed dose of tafenoquine will be used. Patients with acute *P. vivax* malaria who have a G6PD activity ≥70% will be recruited. Although

one year's follow up is needed to capture long latency relapses, these are very unusual in the Southeast Asian region and four months is sufficient to capture over 90% of relapses.

5.2 Scientific Rationale

Tafenoquine, as a single dose regimen, has a tremendous advantage over the longer courses of primaquine needed for radical cure. To maintain this advantage, an efficacious dose must be given. The current data available show that the recommended dose of tafenoquine (300 mg) is probably insufficient in Southeast Asia. This study will assess a higher dose of tafenoquine to identify the optimal dose that can be used for the treatment of *P. vivax* infection and for future malaria elimination efforts.

6. OBJECTIVES

6.1 Study Objectives

The main focus of this study is to determine the optimal dose of single-dose tafenoquine for the radical cure of *Plasmodium vivax* malaria, appropriate for the epidemiological context of Southeast Asia.

6.1.1 Primary Objective

- Determine whether a 50% increase in tafenoquine dose (TQ-higher) provides better efficacy than the currently recommended tafenoquine dose (TQ-current) as measured by the absence of recurrent *P. vivax* infection by 4 months
- Determine whether TQ-higher provides better efficacy to high-dose (7mg/kg total dose) primaquine as measured by the absence of recurrent *P. vivax* infection up to month 4 in Indonesia only.

6.1.2 Secondary Objectives

- Determine whether a 50% increase in tafenoquine dose (TQ-higher) provides greater clearance of sub-microscopic *P. vivax* infection than the currently recommended tafenoquine dose (TQ-current)
- Characterise the safety and tolerability of a 50% higher dose tafenoquine (TQ-higher)
- Determine the predictive utility of day 7 methaemoglobin for tafenoquine radical cure
- Characterise TQ population PK and PD and correlate the *CYP2D6* metaboliser status and genotype with TQ concentrations *in vivo*
- Explore the presence of TQ metabolites *in vivo*
- Characterise the best predictors of recurrence at day 28 and month 4
- Assess the utility of a day 14 and 21 ultrasensitive qPCR parasite quantitation as a predictor of subsequent relapse

- Determine whether TQ-current provides better efficacy to high-dose (7mg/kg total dose) primaquine as measured by the absence of recurrent *P. vivax* infection up to month 4 in Indonesia only.

6.2 Study Endpoints

6.2.1 Primary Endpoint

- Microscopy positive *P. vivax* recurrence after radical cure treatment up to month 4

6.2.2 Secondary Endpoints

- Sub-microscopic *P. vivax* recurrence after radical treatment between day 28 and month 4
- Sub-microscopic *P. vivax* recurrence with very low parasite density after radical cure treatment at days 14 and 21
- Number of serious adverse events (e.g., hospitalisation for symptomatic haemolysis or methaemoglobinaemia)
- Number of adverse events (e.g., gastrointestinal symptoms)
- Methaemoglobin levels measured by pulse oximetry at day 7
- Tafenoquine AUC and $t_{1/2}$ as estimated from plasma tafenoquine concentrations at days 0, 1, 2, and 7, and at monthly visits until month 4
- Plasma tafenoquine concentrations at days 0, 1, 2, and 7, and at monthly visits until month 4
- Tafenoquine metabolites identification in red blood cell and urine at days 1, and 2 or 3.
- The probability of treatment failure as defined by the probability of one or more of the observed recurrences within 4 months being a relapse, using time-to-event and estimated identity-by-descent of recurring parasite genotypes compared to enrolment parasite genotypes
- Microscopy positive *P. vivax* recurrence after radical cure treatment at months 1, 2, 3, and 4Determine whether TQ-higher or TQ-current provide similar efficacy to high-dose (7mg/kg total dose) of primaquine as measured by the absence of recurrent *P. vivax* infection in Indonesia only.

7. STUDY DESIGN

A total of 700 participants (adults and children) will be enrolled and randomised to one of two arms: the currently recommended tafenoquine dose (TQ-current; e.g., 300mg adult dose) or a 50% higher tafenoquine dose (TQ-higher; e.g., 450mg adult dose). Doses will be equal to 100, 150, 200, 300, or 450 mg, given as a single dose (see section 8.3, *p.25* for the description of the study treatment arms). Each arm will have 350 participants, with competitive enrolment across participating countries until the target sample size is reached. The schizonticidal agent used will be determined by the study site (see section 10.2, *p.*3336) for description of schizonticidal treatment). Tafenoquine will be given concomitantly with the schizonticidal agent on the day of enrolment. The participant will be followed

daily until the malaria smear is negative for asexual parasites or until the schizonticidal treatment is completed, whichever occurs last. Follow up will continue on days 7, 14, 21, and 28 then every month until month 4. Participants will be instructed to follow up in between visits if they are feeling unwell. Treatment allocation will be double-blind.

Applicable only for Indonesia: an additional 120 participants (adults and children) will be enrolled and randomised to receive the national standard of care primaquine regimen (PQ7-high; e.g., 7.0mg/kg divided in 7 daily doses.)

7.1 Study Site

This study will take place at 5 main participating countries:

Mahidol Oxford Tropical Medicine Research Unit (MORU), Cambodia – The study sites are located in Pursat province in western Cambodia and Stung Treng province in northeast Cambodia. Both sites are in remote mountainous forested areas. Approximately 100-200 cases of *P. vivax* are reported annually in each province.

Lao Oxford Mahosot Hospital Wellcome Trust Research Unit (LOMWRU), Lao PDR – This study will take place at one or all of the following provinces; Savannakhet, Sekong, and Attapeu provinces, depending on recruitment rates. These southern regions are malaria endemic and populated by multiple ethnic groups. Approximately 100-300 *P. vivax* cases are treated annually at each of the three provincial hospitals where study sites are located.

Mahidol Vivax Research Unit (MVRU), Thailand – Approximately 200 patients with *P. vivax* are treated annually at the two study sites. One site is located in Tak province and the second site is located in Yala province. Potential participants will be recruited from the outpatient departments where patients seek consultation for medical treatment.

Shoklo Malaria Research Unit (SMRU), Thailand – The two study sites are located in Tak province in Thailand and serve populations in both in northwest Thailand and eastern Myanmar. The terrain is a mix of forested mountains and agricultural fields. Rural and remote populations can receive free health care and malaria treatment at the field hospitals. Nearly 400 *P. vivax* cases are treated annually at the clinic sites in Thailand. Malaria surveillance in 2022 already shows increasing vivax cases in eastern Myanmar.

Oxford University Clinical Research Unit (OUCRU), Vietnam – The study site will be at Phuoc Long Medical Center (PLMC) in Binh Phuoc Province, the southwest region of Vietnam. Health stations located in the rural hill-forested areas provide free medical care and referral to the PLMC. Approximately 150 patients with *P. vivax* are treated annually.

Oxford University Clinical Research Unit (OUCRU), Indonesia – The study site is located approximately 1 hour outside Jayapura, Indonesia at the West Arso Primary Healthcare center in

the northeast corner of Indonesian Papua. This is near the border with Papua New Guinea. In 2023, over 7,000 malaria cases were treated and approximately half were caused by *P. vivax*.

7.2 Study Period

Study training and implementation (screening and recruitment) will start as soon as approvals from the Oxford Tropical Research Ethics Committee (OxTREC), and the local Ethics Committee are obtained. Study participant enrollment and data collection is expected to take up to 3-4 years with a 4-month follow-up. There will be an administrative study preparation time of 6 months before the study starts and an additional 6 months after study completion for laboratory and data analysis. The total study duration is expected to be 5 years.

The end of trial is the date of the last follow up visit of the last participant.

7.3 Study Populations

The beneficiaries of this study are individuals (adults and children) with *P. vivax* malaria infection who normally would receive the 14-day course of primaquine for radical cure alongside the schizonticidal treatment. Most of this population live in remote and difficult to reach areas, thus the primaquine course cannot be supervised to completion. It has been well documented that adherence to the 14-day primaquine course is poor. A single dose of tafenoquine can be supervised and will improve the efficacy of radical cure for all patients. Potential participants who present with a history of fever at the outpatient clinics will be recruited from the outpatient setting, provided they meet all the eligibility criteria.

7.3.1 Inclusion Criteria

- Patients with symptomatic *P. vivax* mono-infection as diagnosed by microscopy
- Fever or history of fever in the previous 7 days
- Quantitative G6PD activity ≥70% of the population median
- Weight >10 kg and ≥2 years old*
- Ability to understand the study instructions and provide written informed consent
- Willing to be followed for 4 months

* The Thailand-MVRU site will recruit only participants who are ≥18 years old.

7.3.2 Exclusion Criteria

- Pregnancy
- Lactation
- Hb < 8 g/dL
- Severe malaria
- Blood transfusion in the last 4 months
- Any previous history of a haemolytic event

- History of allergic response to an 8-aminoquinoline or the nationally recommended schizonticide (e.g., chloroquine, artemether-lumefantrine)
- Presence of any condition which in the judgement of the investigator would place the patient at undue risk or interfere with the results of the study (e.g. chronic disease, medications that potentiate or inhibit *CYP2D6* or *CYP2C8* isoenzyme function)

7.4 Sample Size

The trial hypothesis is that the radical cure efficacy of TQ-higher is at least 10% greater than TQ-current. This will be assessed by the absence of recurrent *P. vivax* infection by 4 months. From previous data in the pre-registration trials (22–24), TQ-current had a radical cure efficacy of approximately 74% by 6 months. In short latency settings, over 90-95% of the recurrences occur within 4 months (7,29) thus, the 4-month endpoint should have a high sensitivity. To detect a 10% difference between TQ-current and TQ-higher, account for 25% loss to follow up, and achieve at least 80% power a sample size of about 350 participants in each arm is needed. The sample size calculation as done in Stata 17 using the following command “power two proportions 0.74, diff(0.10)” and was then adjusted for a 25% loss to follow-up. Significance level (alpha) will be set at 0.05 using a two-sided two-sample proportions z-test. Therefore, for the tafenoquine study endpoints a total of 700 participants will be enrolled across 5 countries. Recruitment will be competitive across participating countries.

Applicable only for Indonesia: The PQ7-high arm is added to give the background radical cure efficacy of the national standard of care. PQ7-high has a radical cure efficacy of >95% (41). In Indonesia, the radical cure efficacy of DP + TQ-current was determined to be 21%. We conservatively estimate the radical cure efficacy of DP+PQ-high to be 90% and CQ+TQ-higher to be 75%. To detect a 15% difference between PQ7-higher and TQ-higher, account for 20% loss to follow up, and achieve at least 80% power, an additional sample size of 120 participants for PQ7-higher is needed for a total of 820 participants in the study.

8. STUDY PROCEDURES

Table 2. Study Procedures

Study Procedure		Visit Day										
		Day 0	Day 1	Day 2-3[a]	Day 7 (+/- 3 days)	Day 14 (+/- 3 days)	Day 21 (+/- 3 days)	Day 28 (+ 2 wk /- 3d)	Month 2 (+/- 2 week)	Month 3 (+/- 2 week)	Month 4 (+/- 2 week)	Recurrence*
Clinical	Consent	√										
	Randomisation	√										
	Tafenoquine (TQ) and placebo dose[a]	√										√
	Primaquine (PQ) dose for 7 days[b]	√	√	√								
	Schizonticide dose	√	√	√								√
	Vital signs and physical examination	√	√	√	√	√	√	√	√	√	√	√
	Medication history	√	√	√	√	√	√	√	√	√	√	√
	Adverse event assessment	√	√	√	√	√	√	√	√	√	√	√
	Methaemoglobin level	√	√	√	√	√	√	√	√	√	√	√
	Urine pregnancy test	Urine (minimum 2 ml)										
		√						√	√	√	√	√
		Fresh capillary blood drop (0.02ml)										
	Malaria smear[c]	√	√	√	√	√	√	√	√	√	√	√
		Fresh capillary blood drop (0.01ml)										
	Point-of-care haemoglobin[d]	√	√	√	√	√	√	√	√	√	√	√
		Urine (minimum 2 ml)										
	Urine tests[e]	√	√	√	√	√	√	√	√	√	√	√
Laboratory		Whole blood (2ml adults, 0.5ml children <5years)										
	Complete blood count[f]	√			√	√						√
		Serum (3ml adults, 1.5ml children <5years)										
	Liver function tests[g]	√			√	√						√
	Renal function tests[h]											
		Whole blood (0.01ml)										
	Quantitative point-of-care G6PD test	√			√							
		Whole blood (1ml)										
	CYP2D6 genotyping	√										
		Whole blood (1ml)										
	Parasite genotyping	√										√
		Dried blood spot (DBS-0.05ml)										
	Low volume parasite PCR detection[i]	√			√	√		√	√	√	√	
		Packed red blood cells (pRBC-1ml)										
	High volume parasite PCR detection				√**	√	√					
		DBS	pRBC(1ml)		Dried blood spot (DBS-0.05ml)							
	Pharmacokinetic blood sampling[j]	√	√	√	√			√	√	√	√	√
		Urine (collect all urine during the visit)										
	Pharmacokinetic urine sampling [k]		√	√								

* See section 8.5 for details on recurrence during study follow up

** Indonesia site only

[a] See section 8.3 for dosing details

[b] Indonesia site only: on days 4-6 the study team will directly observe primaquine treatment at participant's home

[c] Daily follow up will continue until the malaria smear is negative at least once. Smears can be made from capillary blood or whole blood.

[d] Haemoglobin will be performed by the point of care HemoCue 301. Haemoglobin test can be from capillary blood or whole blood.

[e] Urine tests will include haemoglobin, urobilinogen, and markers for haemolysis (e.g., CA-1). CA-1 will only be performed until day 28.

[f] Complete blood count will be performed only at sites with access to haematology testing.

[g] Liver function tests include alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total and direct bilirubin concentration. They will be performed only at sites with access to biochemistry testing.

[h] Renal function tests include blood urea nitrogen and creatinine and will be performed only at sites with access to biochemistry testing.

[i] Capillary blood sampling.

[j] For the exploratory analysis of tafenoquine metabolites in red blood cells 1ml of blood will be taken at days 1 and 2 in addition to the capillary blood sampling

[k] Approximately 2ml of urine will be collected

8.1 Recruitment

Potential participants will be recruited from the outpatient setting at the following sites:

- Mahidol Oxford Tropical Medicine Research Unit (MORU), Cambodia
- Lao Oxford Mahosot Hospital Wellcome Trust Research Unit (LOMWRU), Lao PDR
- Mahidol Vivax Research Unit (MVRU), Thailand
- Shoklo Malaria Research Unit (SMRU), Thailand
- Oxford University Clinical Research Unit (OUCRU), Vietnam
- Oxford University Clinical Research Unit (OUCRU), Indonesia

8.2 Screening and Enrolment

This study recruits *P. vivax* patients who seek treatment at public or local health facilities. The malaria diagnosis will be performed by designated staff (such as the research team or government health care staff) at each facility. Patients who are diagnosed with *P. vivax* malaria by blood smears will be invited to participate in the study.

After written informed consent has been provided, the study staff will measure haemoglobin level and G6PD enzyme activity from finger pricked blood, to ensure that the volunteer is eligible to participate in the study.

If volunteers meet the inclusion criteria they will be enrolled in the study. The study staff who have been trained in phlebotomy will draw approximately 7.14 mL of blood (4.14 mL of blood in children <5 years old) (Table 3). The venous blood specimen will be taken using the standard sterile single use venipuncture kit used at each site and blood will be collected in a blood collection tube specified for the clinical laboratory test. The tube will be labelled with the date, time, day, the patient's study code, and a second identifier (ie. patient initials) as is standard for all research specimens. These phlebotomy procedures will be followed for any study-related blood draw.

Table 3. Specimen collection volume for the study

Study Procedure	Day 0	Day 1	Day 2-3	Day 7	Day 14	Day 21	Day 28	Month 2	Month 3	Month 4	Recurrence (if applicable)
Blood draw	7.14 mL	1.03 mL*	1.03 mL*	5.14 mL*	6.08 mL	1.03 mL	0.13 mL	0.13 mL	0.13 mL	0.13 mL	6.08 mL
Blood draw (child<5yr)	4.14 mL	1.03 mL*	1.03 mL*	2.14 mL*	3.08 mL	1.03 mL	0.13 mL	0.13 mL	0.13 mL	0.13 mL	3.08 mL

*For Indonesia blood volume on day 1 to day 3 will be 1.05 ml and on day 7 will be 6.14 mL (3.14 for child less than 5yr).

8.3 Randomisation

Randomisation will be done by sequentially numbered sealed opaque envelopes and prepared by a Clinical Trials Support Group (CTSG) that is not involved with study activities. The CTSG will maintain a log of the study group allocation and control access to the log. The randomised arms will be TQ-current (the currently recommended tafenoquine dose) and TQ-higher (a 50% higher tafenoquine dose than currently recommended). Tafenoquine treatment will be double blinded so all participants will take a study drug with or without matching placebo. Participants will receive a fixed mg dose depending on their weight. The weight bands will be provided in a dosing table (Table 4). Randomisation will be stratified by site.

Applicable only for Indonesia: the randomised arms will include a 3rd arm for PQ7-high.

Table 4. Tafenoquine dosing table

Weight	**Arm 1** TQ-current	**Arm 2** TQ-higher
>10kg to ≤20kg	100 mg	150 mg
>20kg to ≤35kg	200 mg	300 mg
>35kg	300 mg	450 mg

8.4 Direct Observation of Drug Administration

To ensure complete adherence to the prescribed drug regimens, drug administration for the enrolment episode will be directly observed by the study staff when possible. Study staff will provide the participant with the appropriate dosing of the drug for that day and observe the participant taking the drug.

The following procedures are to be performed for each direct observation of drug administration after the first dosing:

- Record adverse events as reported by participant or observed by study staff
- Record vital signs
- Record the date and time of study drug administration

Study staff will review any available laboratory findings prior to drug administration. The investigator will determine if any adverse event results in inability to continue to comply with study procedures or requires hospitalization.

8.5 Follow-up Visits

After signing informed consent on day 0, participants will be scheduled to return to the clinic daily until the malaria smear is negative once or when the schizonticidal treatment is completed, whichever is later. If necessary, the patients will be admitted for supervised treatment until parasite clearance (ie. living very far away). Follow up will continue on days 7, 14, 21, and 28, then months 2, 3, and 4 (total of 10 – 14 visits depending on parasite clearance time). Refer to Table 2, *p.23* for associated study procedures.

Daily visits must occur on the exact day scheduled. The visit windows for the 7, 14, and 21 visits are +/- 3 days, for the day 28 visit + 2 weeks/- 3 days, and for the months 2, 3, and 4 visits are +/- 2 weeks.

The following procedures are to be performed for each visit:

- Medication history and concomitant medication use
- Record adverse events as reported by participant or observed by study staff

- Record vital signs and physical examination
- Collect blood samples for laboratory tests outlined in Table 2, *p.23*.
- Review all available laboratory results

At study exit on month 4, any participant with ongoing, unresolved AEs will be referred to care for appropriate clinical follow-up in the outpatient department. If the participant has a *P. vivax* recurrence, they will be treated as per the randomisation arm and study activities (blood sampling and follow up) will restart as episode 2, day 0. The total study duration will remain the same. This means that the study completion date is 4 months from the date of enrolment for all participants regardless of the number of *P. vivax* episodes.

During the study, there may be real-life event such as changes to national malaria guidelines or participant requests (see Appendix B for details specific for the Indonesia site). Whilst we aim to follow the study protocol, there may be a need to account for these real-life situations. For example, if a participant did not tolerate tafenoquine at enrolment, they or the investigator may refuse tafenoquine treatment at a recurrence. Rather than excluding the participant, primaquine will be provided and follow up will continue.

8.6 Missed Visits

If the participant does not show for a scheduled study visit, study personnel (or community engagement) will call the participant or visit their home to remind the participant of the study visit. Maximum efforts will be made to ensure complete follow-up in the trial, including routine education for participants on follow-up schedules. For participants who do not complete a scheduled visit within the visit window, that visit will be documented as “missed” and follow up will continue.

8.7 Participant Withdrawal or Termination

Each participant has the right to withdraw consent for the study at any time. In addition, the investigator may discontinue a participant from the study at any time if the investigator considers it necessary for any reason including:

- Ineligibility (arising during the study or retrospective having been overlooked at screening)
- Significant non-compliance with treatment regimen or study requirements
- An adverse event which requires discontinuation of the study medication or results in inability to continue to comply with study procedures

The reason for withdrawal will be recorded in the case report form (CRF). If the participant is withdrawn due to an adverse event, the investigator will arrange for follow-up visits or telephone calls until the adverse event has resolved or stabilised. Participants who have been withdrawn or terminated from the study will not be replaced.

8.8 Premature Termination or Suspension of Study

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be promptly provided by the suspending or terminating party to the sponsor, Data Safety Monitoring Board (DSMB), independent ethics committees (IECs).

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable

Study may resume once concerns about safety, protocol compliance, and/or data quality are addressed and satisfy the sponsor and IRBs/IECs.

9. LABORATORY TESTS

All clinical laboratory results will be reviewed by a qualified staff member and recorded in the CRF. Laboratory tests will be performed according to the schedule outlined in Table 2, *p.23* of study procedures. Each of these laboratory tests is described in greater detail below. For all sites, overseas shipping for specimens will be accompanied by Material Transfer Agreement signed by the relevant authorities.

9.1 G6PD Assays

- **Quantitative G6PD test** (10 µL)

  The quantitative assessment of G6PD activity will be performed using the point-of-care STANDARD G6PD Biosensor (SD Biosensor) and is part of screening procedures. User's test proficiency will be tested regularly and monitored by an independent assessor. Test results will be shared with the central haematology laboratory of SMRU at regular intervals in order to identify major deviations from expected performance.

9.2 Haematology Tests

- Point of care haemoglobin (10 uL)

  The HemoCue Hb 301 System provides quick and easy-to-use assessment of haemoglobin concentration from 10 uL of blood collected either by finger prick or by venipuncture. It uses microcuvettes to collect the correct amount of whole blood and measures absorbance by spectrophotometry at an Hb/HbO_2 isobestic point. It reads at two wavelengths (506 and 880 nm) to compensate for turbidity. Cuvettes can be stored at 15-40°C and the analyser can be operated from 10 - 40°C and at 5 - 90% non-condensing relative humidity. The HemoCue system has been shown tropical settings to provide reliable assessment of Hb levels for both screening of anemia and general clinical purposes.

- **Complete Blood Count** (2 mL or 0.5ml in children <5years)

  Whole blood will be collected by venipuncture and placed in an EDTA tube. Samples will be processed and analysed as per the site specific laboratory protocols.
  Leftover blood from the EDTA tube will be aliquoted and stored at -80°C for up to 10 years for future analysis of biomarkers for haemolysis and other assays that increase the understanding of malaria pathophysiology.

- **Methaemoglobin Level Testing**

  Methaemoglobin levels will be measured with a finger sensor using a Masimo Radical 57 oximeter. This procedure is non-invasive.

9.3 Biochemical Tests

- **Liver and kidney function tests** (3 mL or 1.5 ml in children <5years)

  Whole blood will be collected by venipuncture and placed in a plain tube. Samples will be processed (i.e., centrifuged) and analysed as per the site specific protocols.

  Leftover serum will be stored at -80°C for up to 10 years for the future analysis of potential biomarkers for haemolysis and other assays that increase the understanding of malaria pathophysiology.

9.4 Pharmacokinetic Tests

- **Tafenoquine drug levels in blood** (50 µL)

  Blood samples will be obtained by finger prick and spotted onto filter paper (Whatman 3031-915 Grade 31ET Chr Cellulose Chromatography Paper). See Table 2, *p.23* for sample collection time points. The blood spots will be dried and the individual piece of filter paper will be stored with desiccant gel packs until processed at the Faculty of Tropical Medicine, Mahidol University for tafenoquine PK analysis.

- **Tafenoquine metabolite levels in urine** (>2 mL)

  At least 2 ml of urine will be collected for a timed sample on days 1 and 2 for tafenoquine metabolite assessment because the peak plasma concentrations of tafenoquine will occur around 12-20 hours after the dosing. Some participants may have low *CYP2D6* activity. It is not clear whether tafenoquine metabolism is dependent on the *CYP2D6* pathway, therefore the day 2 sample may also be informative. As this aspect of the study is exploratory, the MORU pharmacology lab will develop the laboratory assay for this analysis.

- **Tafenoquine metabolite levels in red blood cells** (1 mL)

  Approximately 1 ml of whole blood will be collected on days 1 and 2 for tafenoquine metabolite assessment. See above paragraph for the justification of the sample timing. This sample will also be utilised for the tafenoquine plasma sampling. As this aspect of the study is exploratory, the MORU pharmacology lab will develop the laboratory assay for this analysis.

9.5 Other Testing

- **CYP2D6 genotyping** (1 mL)

  All patients will be genotyped for *CYP2D6*.

  1 mL of whole blood will be collected at D0 in EDTA tubes, and then it will be kept in -20°C until shipping specimens with dry ice to the molecular malaria lab located in Bangkok. EDTA-treated venous blood will be spun down for 5 minutes at 3000 rpm, and buffy coat and packed

red blood cells (RBCs) will be stored in labeled tubes for later DNA extraction and genotyping of CYP genes according to the manufacturer's guidelines. To genotype *CYP2D6*: firstly, we will determine *CYP2D6* gene duplications/multiplications and gene deletions by extra-long range polymerase chain reaction (XL-PCR) using previous protocol (42). Secondly, we will discriminate of functional *CYP2D6* and nonfunctional *CYP2D8* and *CYP2D7* genes by intron 2 sequencing (INT2). Thirdly, in order to identify *2,*4,*5,*10,*35, and*36 variants, rapid identification of four polymorphic loci by allele-specific oligonucleotide probes real-time SNPs genotyping (43) will be performed. Lastly, in order to identify novel mutations, full length sequence of *CYP2D6* will be amplified in a random selected subset of samples (10-20%) using previous protocol (42).

The *CYP2D6* genotyping will be used to determine the *CYP2D6* activity score.

Malaria smear (20 µL)

A thick and thin malaria smear will be made at each scheduled visit from a finger prick. Thick and thin blood films will be prepared and stained with Giemsa stain as per standard operating procedures. Malaria smears will be declared negative if no parasites are detected in 200 high power fields (H.P.F; 1000 total magnification) on the thick blood film. For positive smears asexual parasites, Schizonts and Gametocytes will be counted against 500 WBC on the thick blood film. For the higher parasitaemia, when the asexual parasites count is more than twice the WBC count on the thick blood film, parasites may be counted on the thin blood film against 1000 RBC. Quality assurance will be performed at SMRU by trained and experienced microscopists.

- **Urine β-human chorionic gonadotropin (HCG) pregnancy test (≥2 mL)**

  A urine β-HCG pregnancy test will be performed in all women of childbearing age (unless menstruating) during initial screening. Bioline HCG test strips or equivalent will be used. After initial screening, the test will be performed during follow up visits (Table 2, *p.23*). Pregnancies occurring after initial treatment with the study drugs will be followed until after delivery to assess for adverse events and delivery outcome. Any study participants identified as being pregnant will be informed and referred for antenatal care.

- **Parasite genotyping** (1mL)

  A whole blood EDTA sample will be collected and frozen at -80°C. *P. vivax* genomic DNA will be extracted using an automated DNA extraction system (QIAsymphony SP (Qiagen, Germany) and QIAsymphony DSP DNA mini kit (Qiagen, Germany) according to the manufacturer's instructions. Initially three polymorphic microsatellite loci will be amplified in paired enrolment and recurrence samples. If two or three of the three loci show evidence of identical by state, six additional microsatellite markers will be genotyped (44). Parasite genotyping is advancing rapidly. Whole genome sequencing using amplicon sequencing will

be considered if the technology is appropriate at the time of sample analysis. This is accounted for in the proposed budget.

- **Low volume quantitative PCR testing** (50 µL)

  A blood spot on filter paper will be collected. The blood spot will be dried then stored with a desiccant in an airtight bag. The sample will be shipped at room temperature to the molecular lab located in Bangkok for later DNA extraction using the commercially available DNA Blood Kit (Qiagen). Semi-nested PCR approach will be used for all fragments. Amplification conditions and primers used will follow standard published protocols (45,46).

- **High volume (ultrasensitive) quantitative PCR testing** (1mL)

  Whole blood EDTA samples will be collected and centrifuged at 2,500 rpm for 10 minutes. The packed red blood cells will be stored at -20°C until specimens are shipped with dry ice to the molecular malaria lab located in Bangkok for later DNA extraction in a methods as previously described (47). The purified DNA will be dried completely in a centrifugal vacuum concentrator and then suspended in a small volume of PCR-grade water to provide a concentrate corresponding to 100 µl of whole blood. 18S rRNA-targeting primers will be used as per the original protocols of Kamau et al. (2011) on serial dilutions from 250,000 to 16 parasites per mL. An estimate of the parasite numbers in each sample will be derived from extrapolation of the threshold cycle (CT) value obtained for the unknown sample to the standard curve using the Rotor-Gene Q series software v. 2.0.2.

- **URICA CA-1 test (1mL urine)**

  Urine carbonic anhydrase-1 (CA-1) is a marker that detects early intravascular haemolysis. It is available as a point of care lateral flow device (similar to a malaria rapid diagnostic test). Three drops of urine will be applied to the lateral flow device. Results will be recorded in the CRF and an image of the result, identified only by the date of testing and Study participant number, will be uploaded to the URICA Study-Image Upload App. Additionally, a 1mL urine sample will be stored at -20°C until specimens are shipped with dry ice to the central MORU laboratory in Bangkok. Urine CA-1 potentially can detect haemolysis earlier than other markers such as haemoglobin, or urine blood and urobilinogen.

10. STUDY DRUGS

10.1 Formulation of Study Drugs

Schizonticidal drugs will be dosed as per the national malaria guidelines in the respective countries.

Applicable only for Indonesia: chloroquine is not currently recommended for treatment of *P. vivax* in the Indonesia national malaria guidelines however, given the INSPECTOR study results (48), alternatives to the combination of dihydroartemisinin piperaquine with tafenoquine are needed.

Cambodia site: Chloroquine is provided as 250 mg tablets (155 mg base). Tablets will be procured from Utopian Co., Ltd. (Thailand) or Remedica (Cyprus, EU).

Lao PDR site: Artemether-lumefantrine (Coartem™) are provided as 20/120 mg tablets and will be procured from Novartis through the National Centre for Malaria Parasitology and Entomology (CMPE).

Thailand site: Chloroquine is provided as 250 mg tablets (155 mg base) and will be supplied through the Global Fund Programme.

Vietnam site: Chloroquine is provided as 250 mg tablets (155 mg base). They will be procured from the Mekophar Pharmaceutical Company through the National Malaria Control Programme.

Indonesia site: Dihydroartemisinin piperaquine is provided as 40 mg dihydroartemisinin and 320 mg piperaquine. Tablets will be provided based on local or regional availability of products. As a second blood schizonticide chloroquine is provided as 250 mg tablets (155 mg base). Tablets will be provided based on local or regional availability of products.

Radical curative treatment:

Tafenoquine will be procured as 100 mg base, coated tablets from Biocelect (Suite 5.02, Level 5, 139 Macquarie Street, Sydney NSW, 2000 Australia). Tablets will be given whole, cut or crushed depending on the dose required. Dosing will be based on weight bands (see section 10.2 below for drug administration and section 8.3, *p.25* for the dosing table). Matching placebo tablets as 100mg will be procured from 60 Degrees Pharmaceuticals (1025 Connecticut Avenue NW, Washington, D.C. 20036 USA).

10.2 Administration of Study Drugs

Depending on the randomisation, the participant will receive a fixed single dose of tafenoquine based on their weight band for radical cure in mg base (for dosing table, see section 8.3, *p.25*):

- Tafenoquine 100 mg single dose
- Tafenoquine 150 mg single dose
- Tafenoquine 200 mg single dose
- Tafenoquine 300 mg single dose
- Tafenoquine 450 mg single dose

Standard schizonticidal treatment will be given concomitantly with the study treatment and will follow the recommended dosing for the site.

Applicable only for Indonesia: standard hypnozoiticidal treatment with primaquine 7mg/kg divided in 7 daily doses will be provided as per the national malaria guidelines.

Table 5. Schizonticidal treatment drug information

Site	Drug name	Drug dosing
Cambodia	Chloroquine	25 mg base/kg divided as: 10 mg base/kg on day 0 and 1, then 5 mg base/kg on day 2.
Lao PDR	Coartem (artemether 20 mg-lumefantrine 120 mg)	Treatment dose for three days for all weight groups. Body weight 5-14 Kg: 1 tab x 2/day, 15-24 Kg: 2 tab x 2/day, 25-34 Kg: 3 tab x 2/day, 35 Kg and above: 4 tab x 2/day.
Thailand (all sites)	Chloroquine	25 mg base/kg divided as: 10 mg base/kg on day 0 and 1, then 5 mg base/kg on day 2.
Vietnam	Chloroquine	25 mg base/kg divided as: 10 mg base/kg on day 0 and 1, then 5 mg base/kg on day 2.
Indonesia	Chloroquine Dihydroarteminisin piperaquine	25 mg base/kg divided as: 600mg on day 0 and 1, then 300mg on day 2; or 10 mg base/kg on day 0 and 1, then 5 mg base/kg on day 2. 3 tablets daily for weight <60 kg for 3 days (day 0, day 1, day 2) or 4 tablets daily for weight ≥ 60 kg for 3 days (day 0, day 1, day 2)

All treatment doses will be supervised. The investigator may withdraw a participant if dose compliance is considered unsatisfactory. If vomiting occurs within 30 minutes of the first dose, the full dose will be re-administered. If vomiting occurs 31-60 minutes of the first dose, half of the dose will be re-administered. The absorption rate constant for tafenoquine was a mean (and also median) of 0.25/hr, derived from nearly 900 patients in Thakkar et al., 2018. Thus 25% of the initial tafenoquine dose was absorbed on average in the first hour. Re-dosing tafenoquine after vomiting using the above thresholds is reasonable.

10.3 Storage of Study Drugs

Storage of study medication will be in accordance with the product label and dispensed by an authorised staff member. Storage temperature and dispensation of study drugs will be recorded.

10.4 Study Drug Accountability

All movements of study medication between pharmaceutical or distributor companies and study sites will be documented. All treatment doses will be observed, therefore, all unused medication at the study sites will be collected and recorded by a member of the Investigator team and discarded.

10.5 Concomitant Medication

If needed, study investigators may prescribe any concomitant medications or treatments deemed necessary to provide adequate care for any adverse event during the study period. Any medication taken during the study will be recorded in the CRF.

11. SAFETY ASSESSMENTS AND REPORTING

11.1 Adverse Event (AE)

An AE or adverse experience is:

Any untoward medical occurrence in a study participant administered a medicinal product, which does not necessarily have to have a causal relationship with this treatment (the study medication).

An AE can therefore be any unfavourable and unintended sign, symptom or disease temporally associated with the use of the study medication, whether or not it is considered related to the study medication. Tafenoquine can cause significant haemolysis in G6PD deficient and intermediate persons. As per product specifications only individuals with 70% or greater G6PD activity will be enrolled in the study, so haemolytic effects are not expected. However, if unexpected haemolysis occurs at any follow up visit to day 28 (or between scheduled visits); or if a study participant is found to be G6PD deficient after tafenoquine dosing, they will be admitted to the hospital and closely monitored for symptoms and haemoglobin recovery. See section 11.3 below for more details.

11.2 Severe Adverse Event (SAE)

A severe adverse event (SAE) is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
- Requires inpatient hospitalisation or prolongation of existing hospitalisation, except for routine hospitalisation for *Plasmodium vivax* or *falciparum* malarial disease
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect

11.3 Expected Adverse Event

All participants will be counselled to return to the health care provider if their symptoms have not improved or if they develop fatigue, difficulty breathing, pallor, feeling of a fast heart beat (palpitations), yellow skin (jaundice), or dark coloured urine (symptoms of anaemia or haemolysis). We do not necessarily expect haemolysis because all participants should have met the inclusion criteria for having G6PD activity ≥70%, as per the product labelling. However, because haemolysis is a significant adverse effect of tafenoquine we plan to train all study staff to recognise the signs and symptoms as listed above and to discuss all symptomatic participants with the clinical researcher site lead. If the haemoglobin has dropped >30% from pre-dose, or absolute Hb is <9 g/L, or if symptoms are present with any haemoglobin drop, the participant will be admitted or referred to the hospital for observation. A medical history and complete physical examination will be performed. Haemoglobin levels and haemoglobinuria will be assessed daily; laboratory tests for haemolysis (e.g., CBC, peripheral smear, liver function tests, LDH, haptoglobin) and kidney function

(ie. BUN, creatinine) will be taken where possible. Haemoglobinopathies and co-morbidities causing anaemia are common in the study areas. Vivax malaria itself also causes anaemia. Thus, we anticipate some study participants will be enrolled with an Hb 8.0 – 8.9 g/dL. These patients will not be automatically admitted for observation (as a result of meeting the criteria of Hb <9g/dL). Instead they will be admitted for observation if signs or symptoms are present with a haemoglobin drop or if the investigator has a concern for the patient's clinical condition.

- If a participant is found to be G6PD deficient (e.g., a female heterozygote) after enrolment and study drug has already been administered, the patient will be hospitalised for observation. Physical examination, symptoms, haemoglobin and haemoglobinuria will be assessed daily; laboratory tests for haemolysis (e.g.. CBC, peripheral smear, liver function tests, LDH, haptoglobin) and kidney function (ie.g., BUN, creatinine) will be taken where possible. Further testing for G6PD genotype and phenotype will also be performed.

Methaemoglobin levels may increase in normal persons taking tafenoquine but as with primaquine, patients are usually asymptomatic. To date, trials assessing single dose tafenoquine show lower peak levels than equivalent primaquine doses. Symptoms of methaemoglobinaemia may consist of cyanosis, lethargy or dyspnea. During study drug administration, symptoms of methaemoglobinemia and methaemoglobin levels (using a Masimo Radical 57 oximetry machine) will be monitored at each visit.

- If a participant develops an increase in SpMet (3-20%) and does not have symptoms, the participant will be monitored using site protocols until levels normalise.
- If a participant develops symptomatic methaemoglobinaemia or if SpMet >20%, the patient will be admitted to the hospital and treated using site protocols.

11.4 Reporting Procedures for Adverse Events

The following information will be recorded: description, date of onset and end date, severity, assessment of relatedness to study medication, other suspect drug or device and action taken. Follow-up information should be provided as necessary.

AEs as judged by a medically qualified investigator will be followed until resolution. All AEs will be managed by the clinical study site team in accordance with good medical practices and the standard clinical practices. The clinical team will assess and treat or refer the participant for medical care as appropriate, which may include additional study visits, if necessary. All related AEs that result in a participant's withdrawal from the study or are present at the end of the study, should be followed up until a satisfactory resolution occurs.

It will be left to the investigator's clinical judgment whether or not an AE is of sufficient severity to require the participant's removal from the study (see Section 8.7). A participant may also voluntarily withdraw from the study due to what he or she perceives as an intolerable AE. If either of these

occurs, the participant will be given appropriate care under medical supervision until symptoms cease or the condition becomes stable.

The severity of adverse events will be assessed following the Common Terminology Criteria for Adverse Events (CTCAE) v 5.0:

1 = mild, 2 = moderate, 3 = severe, 4 = life-threatening, 5 = fatal.

The severity of events will be assessed on the following scale:

- 1 = mild (asymptomatic or mild symptoms requiring clinical or diagnostic observations only; no intervention indicated)
- 2 = moderate (symptoms limit age appropriate instrumental activities of daily living; minimal, local or non-invasive intervention indicated)
- 3 = severe (symptoms are medically significant but not life threatening, disabling, or limit self care activities of daily living; hospitalisation or prolongation of hospitalisation indicated)
- 4 = life threatening (symptoms are life threatening; urgent intervention indicated)
- 5 = death

A medically qualified investigator will assess the relationship of AEs to the study medication. The relatedness of AEs to the study product administration will be assessed on the following scale:

- Definitely related: adverse event and administration of study product are related in time, and a direct association can be demonstrated with the study product
- Probably related: adverse event and administration of study product are reasonably related in time, and the adverse event is more likely explained by the study product than by other causes
- Possibly related: There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of study product). However, the influence of other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events).
- Probably not related: a potential relationship between administration of study product and adverse event could exist, but is unlikely, and the adverse event is most likely explained by causes other than the study product
- Not related: the adverse event is clearly explained by another cause unrelated to administration of the study product. Reportable events must have documentation to support the determination of "not related"

Any pregnancy occurring during the clinical study and the outcome of the pregnancy will be recorded using the pregnancy report form and followed up for congenital abnormality or birth defect.

11.5 Reporting Procedures for Serious Adverse Events

All serious adverse events will be reported to the PI within 1 working day of awareness and to the Ethics Committee as required. SAEs will be reported to the Data Safety Monitoring Board as part of interim or final reports. All SAEs, whether expected or unexpected, will be reported to the DSMB within 2 working days of the PI's notification.

All SAEs must be reported by the site investigator within one day of his or her awareness of the SAE to the Study co-PI and safety team by emailing SAE report to SEADOTsafety@tropmedres.ac. The study co-investigator will report to the PI who will inform the DSMB within 2 working days of initial notification of the SAE and keep the DSMB updated as needed.

SAEs will be followed until resolution even if this extends beyond the study-reporting period. Resolution of an AE is defined as the return to pre-treatment status or stabilisation of the condition with the expectation that it will remain chronic. All SAEs must be submitted as a list every 6 months to the NIH medical officer.

11.6 Safety Oversight (Data Safety Monitoring Board)

Safety oversight will be under the direction of a Data Safety Monitoring Board (DSMB), an independent group of experts that advises the study investigators. The DSMB will be composed of at least three members, including at least one malaria expert and one biostatistician. One of the DSMB members must be a clinician. The primary responsibilities of the DSMB are to 1) periodically review and evaluate the accumulated study data for participant safety and study conduct and progress, and 2) make recommendations concerning the continuation, modification, or termination of the study. The DSMB will operate under the rules of an approved charter that will be reviewed at the organisational meeting of the DSMB held before the study begins recruitment.

The DSMB will meet once approximately half of the target sample size has been enrolled, and then again at the end of the study to assess safety data by randomisation arm. All SAE's and SAR's will be reported to the DSMB within 2 working days of the principal investigator's knowledge of the incident. Prior to each DSMB meeting, a study report will be distributed to the DSMB for review.

12. DATA HANDLING AND RECORD KEEPING

12.1 Data Collection

All study data will be recorded on standard paper Case Report Forms (CRFs). Data will be entered on a secure database in accordance with standard operating procedures (SOPs). A study-specific data management plan will detail procedures for collection, curation and storage of study data.

Source documents are original documents, data, and records from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and

previous and concurrent medication may be summarised into the CRF), clinical charts, laboratory, correspondence, log books, and CRFs. For most variables, the CRF will be considered the source document.

All documents will be stored safely in confidential conditions. On all study-specific documents, other than the signed consent and screening logbook, the participant will be referred to by the study participant number/code, not by name.

12.2 Data Access

The participants will be identified by a study identification number in the study database. The name and any other identifying detail will NOT be included in any study data electronic file. The database linking the volunteer's clinical identification number (ie. hospital number) to the study identification number will be kept by the individual study sites and the data analysis team will not have access to this information. All records will be kept locked and all databases will be password protected such that clinic staff and study staff will have access to their respective databases.

Direct access will be granted to authorised representatives from the sponsor, host institutions and the regulatory authorities to permit trial-related monitoring, audits and inspections.

12.3 Data Storage

Each study site will maintain, and store securely, complete, accurate and current study records throughout the study. In accordance with the University of Oxford's policy, research data and records should be retained for five years after completion of the study for adults, and for children until the youngest child participating in the trial reaches 21, or in accordance with the local/in-country regulation. Applicable records include source documents, site registration documents and reports, informed consent forms, and notations of all contacts with participants.

12.3.1 Data Sharing

Participant's data and results from blood analyses stored in the database may be shared according to the terms defined in the MORU data sharing policy or other researchers to use in the future. All personal information will be anonymised so that no individual can be identified from their treatment records. The genomic data sharing plan will be shared on a public database and the plan will be kept in the Regulatory Binder (or manual of procedures).

12.4 Quality Control and Quality Assurance

The study will be conducted in accordance with the current approved protocol, International Conference on Harmonisation (ICH) Good Clinical Practice (GCP), relevant regulations, standard operating procedures, and the General Data Protection Regulation (GDPR). The Clinical Trials Support Group (CTSG) at MORU will provide study oversight, quality management, and study monitoring.

13. STATISTICAL ANALYSIS

Baseline participant characteristics will be described by treatment arms as either mean and standard deviation, median and interquartile range and/or full range, or expressed as frequency and percentage depending on the data characteristics. We will perform an intention-to-treat (ITT) analysis where participants will be analysed according to the arm of randomisation irrespective of the treatment that was actually given and participant adherence to the study drug. Multiple imputation (MI) will be used for handling missing outcome data each missing value having 5 multiply imputed values that will be combined using Rubin's rule [Rubin DB. 1996]. These analyses will be followed by Complete case (CC) analysis. To assess the differences in the primary and secondary endpoints (microscopy and sub-microscopic *P. vivax* recurrence by month 4, respectively) between treatment arms, generalized linear models for the binomial family with identity link will be used to obtain risk differences and the corresponding 95% confidence interval (CI). A multivariable mixed-effects generalized linear model for the binomial family with identity link will be fitted for the primary outcome. This model will include random intercepts for country and site, and explanatory variables such as demographic factors and baseline characteristics; the schizonticidal drug (chloroquine and artemether-lumefantrine); tafenoquine mg/kg dose, AUC, C_{max}, $t_{1/2}$; potential tafenoquine metabolites; *CYP2D6* status, and methaemoglobin level. Survival analysis will be used to evaluate time to microscopy positive *P. vivax* recurrence. For the adverse event analysis, Fisher's exact test will be used for binary data. Haemoglobin and methaemoglobin levels and association with drug exposure will be analysed using linear regression. The estimated tafenoquine AUC model will be based on previous work on primaquine pharmacokinetic-pharmacodynamic analysis using nonlinear mixed-effects modelling. A detailed statistical analysis plan (SAP) will be developed as a separate document and will be finalised before the database is locked and the details of analysis for the secondary outcomes will be in the SAP.

14. ETHICAL CONSIDERATIONS

14.1 Principles for Clinical Research

The investigator will ensure that this clinical trial is conducted in compliance the International Conference on Harmonization Good Clinical Practice E6 (R2) (ICH-GCP) or the Declaration of Helsinki, whichever affords the greater protection to the participant.

14.2 Community Engagement

Thailand site: The populations served are predominantly migrant workers, refugees and ethnic minorities. Most live in remote villages which means the population has limited access to health care. Health care services are provided in addition to malaria diagnosis and treatment. Staff that specialise in community engagement have previously held activities in the region, which include discussions and theatre on topics such as malaria, G6PD deficiency, or other relevant problems

affecting health. This means that the research teams already have connections to and trust with the communities. Engagement with the community leaders, village members and a recently formed Youth Persons Advisory Group (YPAG) will continue during this study.

Consultation with the Community Advisory Board of Tak Province will be undertaken for the SMRU site. This Board comprises representatives from the communities that SMRU serves. Study objectives and procedures will be presented (with translation as necessary into Karen or Burmese) to the Community Advisory Board and discussed in detail.

Lao PDR site: Before the study starts, consultation meetings will be organised to discuss about the study with provincial and district health leaders and technical staff in the malaria division. Following the consultation meetings, detailed activities of the study will be planned for the implementation. Later, the district health office will issue official letters to be sent to each village head to inform about the study and asking the patients with vivax malaria to participate in the trial. The village heads will then meet with their villagers to inform them about the study.

Cambodia site: Both Cambodia sites have strong collaboration with the local authorities (e.g governor's office and health department). The teams have experience in conducting community-based engagement activities (e.g. Village Drama Against Malaria, School Engagement etc.), which were performed at villages in Siem Pang District. There is an ongoing trial on Forest Goers prophylaxis which involves engaging Community Health Workers, Village Leaders, villagers and local authorities in Siem Pang District. Similar activities will be conducted in this trial to increase awareness of *P. vivax* malaria.

Vietnam site: After local ethics approval, the study team will start the protocol training for the community engagement teams. The Director of provincial hospital and Head of Health Station will be invited to participate into the training. Before study implementation, all the public as well as private health care sectors will be informed about the trial. The community health workers work closely with study team staff and health station to inform and screen any eligible volunteers.

Indonesia site: Review of the master protocol involves collaboration with the Indonesia RA (BPOM-Indonesian Food and Drug regulatory body), National Malaria Control Programme (NMCP)-MoH, Chief of District Health Keerom, Chief of Arso Barat PUSKESMAS, and clinical staff of PUSKESMAS (government community health clinics). Where resources allow, members of a community advisory group (CAG) will be enlisted to interact directly with patients to promote discussion around this study.

14.3 Informed Consent

Clinic staff assigned to study duties will meet individually with the volunteer in a private space and review the study details, using the Participant Information Sheet and Informed Consent form to help summarise the main points of the study. They will emphasise that participation in this study will be voluntary, and under no circumstances will the clinical management of the volunteer be affected by

their decision to participate or not in the study. Following summary of the study, the study staff will review the Participant Information Sheet (PIS) and Informed Consent Form (ICF) with the potential participant (volunteer). As per local ethics committee requirements, children will be asked to sign or affix a thumbprint on an assent form. The PIS-ICF (and assent if required) will review the study purpose, the procedures involved, the volunteers' rights to withdraw, confidentiality, and benefits and risks of participating in the study. Volunteers will be given the opportunity to ask questions about the study, procedure, and the consent process. Volunteers will be consented using a PIS-ICF (and assent if required) in the language which they understand; the PIS-ICF (and assent if required) will be prepared in English and translated into local languages.

Volunteers will be asked to sign the ICF (and assent if required) as a confirmation of willingness to enroll in the study. Volunteers who are unable to read and write will be provided an independent witness who will attend the briefing session and sign and date the consent form prior to entry of the volunteer to the study.

If an amendment to the study protocol is submitted and approved, the PI will ensure that the PIS-ICF is revised to reflect changes relevant to the volunteer's participation in the study. Any modification to the ICF will be approved by the appropriate ethics committees before being used with participants.

14.4 Participant Confidentiality

The investigator will ensure that each participant's confidentiality is maintained. Participants will not be identified in any publicly released reports of this study. All records will be kept confidential.

Clinical data will be recorded on a Case Report Form with only the study participant number (e.g. a study code). The patient's hospital number will be entered into the source document and will not be entered into the CRF.

14.5 Benefits & Risks

14.5.1 Benefit to study participants

Study participants may benefit from this study by receiving radical curative treatment for their *P. vivax* infection. Tafenoquine, unlike primaquine, is administered as a single dose which improves treatment adherence to radical cure treatment and consequently reduces the risk of relapse. Low dose tafenoquine has already proven to have ~60% efficacy against *P. vivax* recurrences, thus some of the participants randomised to the lower tafenoquine dosing arms will still achieve radical cure of their *P. vivax* infection.

14.5.2 Benefit to participating study sites

By reducing *P. vivax* recurrences, transmission is reduced. This benefits the villages surrounding the clinic as well as the villages where the study participants live. Children in this region will benefit

disproportionately because *P. vivax* infection occurs more frequently in children in low transmission settings, such as SE Asia. Providing efficacious treatment and improving the treatment given to patients who have previously been seen at the clinic may help strengthen the relationship of the clinic with its community members. In addition, the training and monitoring that will accompany the introduction of the study SOPs may improve the overall technical capacity at the sites.

14.5.3 Risk Considerations

Pregnant or lactating women are excluded from this study. Only persons weighing >10kg will be included, and the minimum age of inclusion will be approximately 2 years. This means a vulnerable population will be included in the study. The study sites already manage and treat malaria in patients in this age group and enroll children in clinical trials. Thus, they understand community perceptions of malaria treatment in children and experience with parental views when enrolling children in malaria specific clinical trials.

Tafenoquine has a similar side effect profile with primaquine, which means generally it is well tolerated. Tafenoquine causes a dose dependent haemolysis in persons with intermediate and deficient G6PD activity (13,32). It should not be given in pregnant women (foetal G6PD status unknown) or during lactation (for G6PD deficient infants) and these populations are excluded from this study. No serious adverse events related to haemolysis have occurred in G6PD normal individuals with ≥70% G6PD activity, thus quantitative G6PD testing is required to determine eligibility in this study. There is no convincing evidence that tafenoquine causes neuropsychiatric toxicity (49). Psychiatric symptoms have been associated with the higher causal prophylaxis tafenoquine regimen (daily and weekly doses) not the single dose radical curative regimen as in this study and is not a contraindication in the product labels. Tafenoquine causes dose dependent reversible changes in the ocular tissue (vortex keratopathy) that do not generally cause vision changes (50–52). Thus, single dose regimens are unlikely to cause pathologic changes in the eye. Electrocardiographic (ECG) changes with tafenoquine have been well evaluated; no clinically significant ECG changes have been observed comparing tafenoquine and placebo (53,54). Sudden unexplained deaths have not been reported with tafenoquine use. Abdominal pain is a common adverse effect and is alleviated if the dose is taken after food. Nausea or vomiting is observed to occur more frequently with tafenoquine when compared to placebo.

Chloroquine in combination with tafenoquine is not associated with persistent changes in tafenoquine blood plasma concentrations or clinically significant adverse effects when compared to tafenoquine alone (55–57). Artemether-lumefantrine co-administration has no effect on tafenoquine blood plasma concentrations (55).

Study procedures do not represent significant risks to the participants beyond those that are associated with normal blood draw, such as pain, discomfort, and infection at the site of finger prick or venepuncture. Participants' blood samples will be collected by well - trained medical personnel

with aseptic technique to prevent infection or complications. During any blood taking procedure, if there is discomfort or pain beyond what is acceptable to the participant, parent or guardian, the procedure will be stopped and no further attempts will be made at that scheduled visit. In children, a standard limited number of attempts for venous sampling will be allowed; this threshold will depend on the study site and local acceptance of blood sampling.

There are additional risk considerations for infection prevention of new emerging infections (e.g. COVID-19). National guidelines will be followed for all in person research activities. Study sites will be required to follow standard operating procedure on the use of personal protective equipment, how they will minimise infectious transmission risk, and the steps they will take to manage persons with symptoms suggestive of new emerging infections. Local and government guidance for face to face research will be followed.

14.6 Compensation

Participants will be compensated for the time/labor costs of participation (Table 6); this is equivalent on average to one day's wages in each study area. Transportation costs will be provided; the amount will be determined by the current transportation rates and will cover transportation costs for most of the catchment areas. The amount to be compensated will be stated in the Participant Information Sheet. Due to changing petrol costs, there is some uncertainty around participant transportation costs and this will be adjusted accordingly as the study progresses.

Table 6. Participation compensation and transportation by site

Study site	Compensation (per screening and study visit)	Transportation (round trip per screening and study visit)
Cambodia	10 USD	10-20 USD
Lao PDR	100,000 LAK (~8 USD)	May be up to 200,000 LAK (up to 16 USD)
Thailand - SMRU	300 THB (~10 USD)	Actual cost up to 600 THB (up to 20 USD)
Thailand – MVRU	600 THB (20 USD)	200 THB (~7 USD)
Vietnam	500,000 VND (~22 USD)	500,000 VND (~22 USD)
Indonesia	IDR 200,000 per day of admission, IDR 100,000 per day of in-clinic visit, IDR	NA

Study site	Compensation (per screening and study visit)	Transportation (round trip per screening and study visit)
	50,000 per day of home visit (~12 USD)	

Note: The compensation may be adjusted according to local guidelines

Patients who do not agree to participate in the study will not be provided compensation. If the patient withdraws after consent, he or she will still be compensated as above for that visit.

14.7 Ethical Review

The protocol, informed consent form and participant information sheet will be submitted to Oxford Tropical Research Ethics Committee (OxTREC) and local independent ethics committees for written approval. Site lead and site co-investigators will submit this master protocol (and modified as necessary) to their local ethics committees.

14.7.1 Amendments

All amendments and modifications to the protocol, ICF, participant information sheet, and will be submitted to the above IECs for review and approval. No changes in protocol conduct will be implemented until approvals by all applicable IECs are obtained.

14.7.2 Continuing Review Reports

The PI will be responsible for submitting the required continuing review report and associated documents to the relevant IECs, allowing sufficient time for annual review and continuation determination prior to the established continuing review date. The end of the study report will be submitted at the end of five years, or upon completion of the study, whichever comes first.

14.7.3 Deviations

Any deviation from the protocol that may have an impact on the safety or rights of the participant or the integrity of the study will be reported relevant IECs according to local requirements. All other deviations will be reported in the annual continuing review report or as per local IEC requirement.

15. FINANCING AND INSURANCE

This project is funded by the National Institutes of Health (NIH) for a duration of 5 years (Sept 2023 to Aug 2028); funding awards are issued annually and are contingent on the work completed in the previous year. The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London).

16. CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the trial. The study leadership has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

17. PUBLICATION POLICY

All data will be published in the open medical literature with the identity of the participant protected. This trial has been registered in a web-based protocol registration scheme at http://www.ClinicalTrials.gov. (ClinicalTrials.gov number NCT04704999)

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19. APPENDIX A: ETHICS COMMITTEES DETAILS FOR SITES OUTSIDE THAILAND

Reviewing Independent Ethics Committees (IECs):	Cambodia site: National Ethical Committee for Health Research Lot#80, Samdach Penn Nouth Blvd (289), Sangkat Moeungkok2, Khan Tuol Kork, Phnom Penh, Cambodia Tel: (855-012) 842 442 http://nechr.org/beta/camhrp/index.php/camhrp Lao PDR site: Ministry of Health National Ethics Committee for Health Research Vientiane, Lao PDR Vietnam site: The Ministry of Health Evaluation Committee on Ethics in Biomedical Research 138A Giang Vo Street, Ba Dinh District, Ha Noi, Viet Nam Email: iecmoh@gmail.com Tel: +84 4 6273 2156 Indonesia site: Health Research Ethic Committee Faculty of Medicine, Universitas Indonesia – Dr. Cipto Mangunkusumo Address: Jl. Salemba 6, Jakarta Pusat Email: ec_fkui@yahoo.com Tel: +62 21 315 7008

20. APPENDIX B. MANAGEMENT OF P. VIVAX AT THE INDONESIA SITE

Parasite clearance during the acute *P. vivax* malaria infection

All enrolled participants will be admitted for twice-daily malaria smears and to assess clinical response.

If chloroquine is used as the blood schizonticide, rescue treatment with dihydroartemisin-piperaquine (DP) according to the standard doses in the national malaria guidelines will be provided in the following situations:

- If the participant's clinical condition is worsening
- If the parasitaemia is increasing during the acute treatment phase
- If parasites (by microscopy) have not cleared by the 7th day of treatment

A dried blood spot for chloroquine and plasma sample for primaquine blood concentration levels will be taken before DP and primaquine are started. The participant will continue to be followed as per the study protocol.

P. vivax malaria recurrence

- If there is a *P. vivax* recurrence ≤28 days, primaquine will be given in combination with DP. Primaquine dosing will follow the national treatment guidelines.
- If there is a *P. vivax* recurrence >28 days, the study protocol will be followed.

The participant will be followed, and study activities will be conducted as per the protocol.

Chloroquine failure (blood stage infection ≤28 days)

- If 2 or more treatment failures occur within 28 days in the first 20 participants, the principal investigator and co-investigators will direct a meeting with the DSMB to examine the evidence and determine a course of action, The DSMB members have discussed and approved this procedure. If the DSMB recommend using DP instead of CQ, the blood stage treatment will change from CQ to DP as per Table 5 (p. 36) to allow for operational realities while reaching study objectives.

Notes:

- The randomisation is independent of the blood schizonticide, so the study may continue without interruption.